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PRIMARY RESEARCH PAPER

# Impact of habitat diversity on the sampling effort required for the assessment of river fish communities and IBI

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**Abstract** The spatial variation in the fish communities of four small Belgian rivers with variable habitat diversity was investigated by electric fishing to define the minimum sampling distance required for optimal fish stock assessment and determination of the Index of Biotic Integrity. This study shows that the standardised sampling distance of 100 m was not always sufficient to collect most species present. The required minimum sampling distance seems to be correlated with habitat diversity. In homogeneous streams, a mean sample distance of 282, 452 and 572 m is necessary to capture 80, 90 and 95% of all species present, respectively. In heterogeneous streams, these sample distances decrease to 217, 380 and 503 m. Hence, at least 300 m should be sampled to catch most species present with a single-pass sampling method. However, our

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results show that a 100 m sampling distance as presently used in the Flemish monitoring programs is sufficient to accurately describe the ecological quality since differences in IBI evaluation between adjacent stretches could at least for some rivers be explained by differences in habitat heterogeneity.

**Keywords** Flanders · Biodiversity · Pisces · Sampling distance · Fishing protocol · Index of Biotic Integrity

## Introduction

Recently, field sampling of resident biological communities has become a primary component of water quality evaluations in Europe. The European Water Framework Directive (WFD; Directive 2000/60/EC, 2000) requires monitoring the ecological quality of water bodies by means of monitoring of biological quality elements. Biological monitoring is defined as the use of a biological entity as detector and its response as a measure to determine environmental conditions (Karr, 1993). The health of fish communities is a sensitive indicator of direct and indirect stresses on the entire aquatic ecosystem. Therefore, fishes are one of the biological quality elements to be monitored to determine trends in the ecological status. Ambient biological monitoring has several major advantages, such as its sensitivity to a broad range of degradation in both water and habitat quality, it integrates cumulative impacts from point and non-point sources, and it can be used to assess trends in space or time (Karr, 1993). The Index of Biotic Integrity (IBI) was developed to assess the ecological quality of lotic systems (Karr, 1981; Karr et al., 1987). It has become a widely used tool for assessing the status of stream fish communities and the overall ecological status of streams (Breine et al., 2005) because it is assumed that fish communities do reflect watershed conditions (Fausch et al., 1990; Hughes and Oberdorff, 1999).

In Flanders, the northern part of Belgium, a modified index of the original IBI (Karr, 1981) has been developed (Belpaire et al., 2000). The Flemish IBI is a composite index, comprising metrics that reflect structural and functional characteristics of fish communities. The IBI integrates a variety of metrics, such as total number of species, relative abundance measures, trophic composition measures and several other metrics (such as natural recruitment and biomass) into a single quality value. Currently, the sample length to assess the biotic integrity in Flanders is set on 100 m for small brooks and rivers (wetted width <10 m), and 2 × 250 m along both river banks for larger rivers. Although, never scientifically founded, the results of the standardised sampling protocol for small wadable streams were presumed to allow evaluating changes in fish communities. However, different studies demonstrated that sampling a 100 m river stretch is not always sufficient to account for discontinuity in fish composition (Angermeier & Smogor, 1995; Paller, 1995; Patton et al., 2000; Teels, 2003). In order to accurately estimate the total number of species in a river zone the minimum sample lengths varied between 200 m (Patton et al., 2000), 271 m (Lyons, 1992) to 235–555 m (Paller, 1995). Angermeier & Smogor (1995) showed that 90% of the species present were usually found by sampling a stream length between 22 and 67 times the stream width.

The estimates of species composition and proportional abundances do not only depend on regional or local differences, but are also very sensitive to sampling efforts and used techniques. In addition, factors, such as sample length (Karr et al., 1986; Didier & Kestemont, 1996), fish movement (Stott et al., 1962; Bruylants et al., 1986; fish migration as well as daily activity patterns) and microhabitat distribution (Lyons, 1992; Angermeier & Smogor, 1995; Didier & Kestemont, 1996) can have a major impact on the catch, and thus on subsequent river quality evaluation. Therefore, observed patterns or changes in fish communities could be biased by inaccurate sampling methodology, being an artefact of the sampling area rather than changes in fish communities per se. Bearing this in mind, it is crucial to guarantee the statistical validity of the sampling methodology.

This study has two major objectives: (1) it is essential that the species composition in the catch reflects the natural species diversity in the river zone. Sampling effort should be sufficient to estimate the fish community as precisely as possible because species richness is one of the eight metrics used for the calculation of the ecological quality of Flandrian river habitats corresponding to the bream or barbel zone. Moreover, species composition is also an important key factor affecting several other IBI metrics

(tolerance classes, trophic composition, presence of exotic species, typical species and type species). Therefore, the calculation of the IBI requires an adequate sampling of the fish community, and all species should be captured in proportion to their true relative abundance (Fausch et al., 1990). In this article, we will only focus on species richness as this is one of the main driving forces of the IBI evaluation. We will evaluate if sampling a river length of 100 m is sufficient to correctly estimate the total number of species or the overall stream fish community. In addition, the hypothesis that a 100 m sample could accurately reflect the local IBI was tested.

(2) Species richness, persistence and assemblage stability might vary considerably dependent on habitat diversity. Hence, accurate estimate of the total number of species might require different sampling efforts in homogeneous versus heterogeneous river stretches. Fish communities of four rivers with varying anthropogenic impact (and consequently varying habitat diversity) were compared to test if different sampling efforts are required.

The findings of this study are relevant to determine the appropriate sampling effort for characterising stream fish communities. Recommendations for appropriate sampling effort in wadable streams are made for use in large scale monitoring projects as prescribed in the Water Framework Directive.

## Materials and methods

### Study sites

Fish community was assessed in 1 km-zones in four small lowland rivers situated in the Scheldt catchment in Flanders (Belgium). The rivers Desselse Nete, IJse, Witte Nete and Kleine Gete were selected based on their permanent good water quality and a (for Flanders) relatively high species diversity. The study sites were comparable in respect to mean breadth (min 4.3 m–max 7.1 m), mean overall depth (min 0.49 m–max 0.61 m) and mean surface water velocity indicatively measured as the mean time necessary to cover a distance of 10 m using a standardised floater (min 0.37 m/s–max 0.55 m/s). Descriptive site measurements were carried out at low flow. The Desselse Nete and the Witte Nete are situated in the bream zone, the Kleine Gete and the IJse in the barbel zone as defined by Huet (1954, 1962). Specific features regarding stream width, depth and heterogeneity between the different sample stretches are shown in Table 1.

### Fish sampling

Standardised electric fishing procedures are described in the CEN directive (CEN, 2003). In our study, electric fishing was conducted by wading using a generator-powered unit (Electracatch Pulsed and smooth DC WFC7) set on low voltage (100 V smooth direct current), with a fixed cathode and one 2 m anode pole (32 cm diameter anode ring). Depending on river width, two different sampling methods were used. Electric fishing in the Desselse Nete (April, 2001), IJse (April, 2001) and the Kleine Gete (May, 2001) was conducted using a 100 m cable. The Witte Nete (April, 2001) was sampled by wading using two 2 m anode poles, whilst pulling the electric fishing gear in a small boat.

In order to minimise the flight bias, which may cause displacement of individuals from their original position, we used a modified point single-pass electric fishing procedure, which permits a random sampling (Van Liefferinge et al., 2005). The activated anode was submerged for several seconds every 0.75 m. Electric fishing is conducted in a zigzag pattern, whilst moving in an upstream direction, carefully sampling all microhabitats.

In each river, seven 100 m sample sections (=river stretches, RS) were separated by 50 m buffer zones to minimise migration between adjacent stretches covering in total a river length of 1,000 m (=river zone, RZ). Effort was made to sample the entire river stretch, and sampling effort between stretches of a river zone was kept constant. Sampling of seven consecutive river stretches in one river zone was completed within 2 days. At each stretch, the stunned fishes captured with a fine meshed dip-net were immediately placed in a large tub. All specimens were counted and identified on species level, measured to the nearest millimetre fork length or total length (first 50 specimens) and weighed to the nearest decigram, before being returned unharmed to the water. If more than 50 specimens were captured, the total biomass per species of the additional number of specimens (group weight) was recorded. The data of the fish stock assessment was used to calculate the IBI as described by Belpaire et al. (2000).

**Table 1** Specific features of the seven reaches at each river site including heterogeneity measurements on macro- and microhabitat scale: habitat structure (HS based on meandering, pool-riffle pattern and the presence of natural hiding places) and depth diversity ($CV_{DD}$)

| Stream/Reach | Depth (cm) | | | | Breadth (m) | Surface stream velocity (m/s) | HS (−6 to +6) | $CV_{DD\ cum}$ | Class |
|---|---|---|---|---|---|---|---|---|---|
| | Min | Mean | SD | Max | Max | | | | |
| DN 1[a] | 23 | 53 | 19 | 117 | 4.0 | 0.52 | 4.0 | 2.327 | Heterogeneous |
| DN 2 | 26 | 46 | 16 | 113 | 5.0 | 0.32 | 3.5 | 2.011 | Heterogeneous |
| DN 3 | 26 | 48 | 17 | 113 | 4.0 | 0.40 | 4.0 | 2.065 | Heterogeneous |
| DN 4 | 26 | 52 | 19 | 101 | 4.5 | 0.44 | 2.5 | 2.126 | Heterogeneous |
| DN 5 | 23 | 51 | 18 | 103 | 5.0 | 0.38 | 3.5 | 1.927 | Heterogeneous |
| DN 6 | 25 | 49 | 16 | 95 | 4.0 | 0.55 | 2.0 | 1.904 | Intermediate |
| DN 7 | 24 | 49 | 25 | 151 | 5.0 | 0.28 | 3.0 | 2.889 | Heterogeneous |
| IJ 1[b] | 33 | 48 | 17 | 105 | 4.5 | 0.58 | 0.0 | 1.894 | Intermediate |
| IJ 2 | 30 | 43 | 13 | 72 | 3.8 | 0.53 | 3.0 | 2.100 | Intermediate |
| IJ 3 | 32 | 49 | 17 | 97 | 4.0 | 0.43 | 0.0 | 2.353 | Heterogeneous |
| IJ 4 | 24 | 46 | 17 | 95 | 4.5 | 0.45 | 2.0 | 2.293 | Intermediate |
| IJ 5 | 30 | 48 | 16 | 94 | 5.0 | 0.32 | 4.0 | 2.167 | Intermediate |
| IJ 6 | 34 | 50 | 18 | 105 | 3.0 | 0.52 | 1.0 | 2.281 | Heterogeneous |
| IJ 7 | 13 | 59 | 30 | 119 | 5.0 | 0.25 | 5.0 | 2.823 | Heterogeneous |
| WN 1[c] | 25 | 36 | 13 | 78 | 7.5 | 0.45 | −3.0 | 1.993 | Homogeneous |
| WN 2 | 23 | 32 | 6 | 50 | 7.5 | 0.31 | −2.5 | 1.425 | Homogeneous |
| WN 3 | 24 | 39 | 8 | 75 | 7.5 | 0.26 | −3.0 | 1.471 | Homogeneous |
| WN 4 | 14 | 39 | 7 | 56 | 7.0 | 0.39 | −3.0 | 1.228 | Homogeneous |
| WN 5 | 32 | 47 | 6 | 65 | 7.0 | 0.43 | −3.0 | 1.106 | Homogeneous |
| WN 6 | 25 | 56 | 17 | 113 | 7.5 | 0.48 | −2.5 | 2.204 | Homogeneous |
| WN 7 | 40 | 57 | 13 | 113 | 6.0 | 0.29 | −2.0 | 1.735 | Homogeneous |
| KG 1[d] | 36 | 61 | 14 | 100 | 6.1 | 0.54 | −1.0 | 1.825 | Intermediate |
| KG 2 | 32 | 53 | 15 | 94 | 6.0 | 0.74 | 0.0 | 1.837 | Intermediate |
| KG 3 | 37 | 62 | 12 | 84 | 5.8 | 0.54 | 2.0 | 1.592 | Intermediate |
| KG 4 | 41 | 78 | 24 | 118 | 5.8 | 0.57 | 1.0 | 1.781 | Intermediate |
| KG 5 | 37 | 55 | 11 | 85 | 6.5 | 0.65 | 0.0 | 1.398 | Intermediate |
| KG 6 | 24 | 58 | 19 | 95 | 5.8 | 0.45 | 3.0 | 1.921 | Heterogeneous |
| KG 7 | 29 | 59 | 23 | 124 | 6.6 | 0.39 | 2.0 | 2.348 | Intermediate |

*DN* Desselse Nete, *IJ* IJse, *WN* Witte Nete, *KG* Kleine Gete

[a] The Desselse Nete has a high degree of habitat diversity, with well-developed pools, riffles and meanders. The river is bordered by meadows and lacks riparian forest. The predominant substrate is sand. Instream structure consists mainly of aquatic macrophytes, and to a lesser extent logs and branches

[b] The IJse also has a good habitat quality, but lacking any riparian vegetation in the first sample section. In the following sections, woodland bordered the right bank. The substrate consists mainly of gravel and stones, which may be serving as instream cover. Macrophytes are scarce

[c] The Witte Nete is straightened, with a uniform and poor habitat quality. It is situated in an agricultural landscape, flanked by meadows and cornfields and lacking any riparian vegetation. The predominant substrate is sand. Instream structure consists mainly of aquatic macrophytes

[d] The Kleine Gete is straightened, with a more or less uniform and poor habitat quality. The substrate consists mainly of gravel and stones, which may be serving as instream cover. Macrophytes are present

### Heterogeneity

Macrohabitat characteristics for each 100 m stretch were assessed based upon three general features of structural diversity: meandering, pool-riffle structure and the presence/absence of hollow banks, each classified by experts' eye as irreparable/absent (−2), absent (−1), weakly developed (0), well-developed (+1) or natural characteristics (+2) (=habitat structure, HS) resulting in scores of −6 to +6.

Furthermore, variability of water depth (depth diversity, DD) was used as a measure of heterogeneity to take into account microhabitat diversity. In each stretch, water depth was measured to the nearest 0.5 cm at equidistant transects (every 5 m) perpendicular to the flow, at five equally spaced points along each transect (one near each bank, one in the centre of the channel and two in between). In the Desselse Nete, sometimes one additional transect was measured when an inner bend of a meander was missed using the measuring protocol. It was important to include these measurements not to misinterpret habitat diversity of the river stretch. In total, there were 21 or 22 transects with five samples, resulting in 105 or 110 data points for habitat diversity per stretch.

In order to determine differences in depth diversity, the coefficient of variance was used (CV equals the standard deviation divided by the mean). The sum of the individual CV of each row (right bank, centre of the channel, left bank and both in between) was calculated, to take into account severe differences in depth profiles caused by local sedimentation and erosion processes as it is the case in small, wandering rivers ($CV_{DD\ cum}$).

### Data analysis

Differences in heterogeneity between rivers and the effect of heterogeneity on the proportion of locally 'rare' species (defined as less than three specimens present in a given river stretch) are tested using a one-way analysis of variances (one-way ANOVA). The groups were checked for normality using a Shapiro Wilks W test. Post-hoc comparisons were determined by a Tukey's post-hoc test or unequal N HSD post-hoc test (Spjotvoll/Stoline test).

Correlation matrices and a within group correlation test was used to determine correlations between habitat diversity and other parameters, such as number of species, IBI score and the percentage of total species caught in the river stretch (%NSP defined as 100 * $NSP_{RS}/NSP_{RZ}$).

'Joining' (tree clustering) was used to visualise similarities and dissimilarities across rivers and in between stretches of a river for (1) lumping heterogeneity measurements on macro- and microhabitat scale: habitat structure (HS) and depth diversity ($CV_{DD\ cum}$) with the weighted pair-group average method and (2) fish communities using the single linkage method. Analysis of species composition was not only based on presence/absence of species but also specific proportions of species were taken into account. All statistics were conducted with Statistica (work package 6.0).

Species richness in each river zone ($NSP_{RZ}$) was determined by pooling the data from all seven river stretches ($NSP_{RS}$). The sampling effort needed to collect 80, 90 and 95% of the $NSP_{RZ}$ was estimated, using a randomisation macro in Microsoft Excel 2000. In order to derive the average fraction of the $NSP_{RZ}$ caught by fishing on 1, 2,… transects, a re-labelling procedure was followed. A small algorithm to construct all (5,040) permutations of the seven stretches was written. For each permutation, the number of species found in RS 1 only, RS 1 and 2, RS 1–3, etc., respectively, were counted. Averaging these numbers over all permutations gives the average fraction of $NSP_{RZ}$ when randomly selecting 1, 2,… river stretches. Consequently, the number of stretches required to catch the different proportions of total occurring species for every river could be assessed and as such the sampling distance necessary to obtain the target level.

## Results

### Habitat and depth diversity

In order to detect differences in habitat diversity between the four rivers, a one-way ANOVA was conducted for $CV_{DD\ cum}$ ($W = 0.97$) and HS ($W = 0.91$; Table 2). The Witte Nete appears to be more homogeneous than the IJse and the Desselse Nete (for $CV_{DD\ cum}$) as expected. No further significant differences could be found, although there is a trend suggesting that the Kleine Gete is more homogeneous

**Table 2** Differences in habitat diversity on microhabitat (depth diversity, $CV_{DD\ cum}$) and macrohabitat scale (habitat structure, HS) between the four river zones (NS* $P < 0.1$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$)

| | Desselse Nete | | IJse | | Kleine Gete | | Witte Nete | |
|---|---|---|---|---|---|---|---|---|
| | $CV_{DD\ cum}$ 2.179 | HS 3.00 | $CV_{DD\ cum}$ 2.273 | HS 2.14 | $CV_{DD\ cum}$ 1.815 | HS −2.74 | $CV_{DD\ cum}$ 1.594 | HS 1.00 |
| Desselse Nete | – | | | | | | | |
| IJse | NS | NS | – | | | | | |
| Kleine Gete | NS | *** | NS* | *** | – | | | |
| Witte Nete | * | * | ** | NS | NS | *** | – | |

than the IJse. Taken into account the macrohabitat results (HS; Table 2), the Desselse Nete and the IJse are grouped as heterogeneous rivers and the Witte Nete and the Kleine Gete are grouped as homogeneous rivers.

Based on Fig. 1, the different stretches could be divided in three major groups (for abbreviations see Table 1): the homogeneous group (from WN 7 to WN 1), the intermediate group (KG 1–DN 6) and the heterogeneous group (IJ 7–DN 1). It seems that the IJse is not as heterogeneous as first presumed. Here, the river zone consists of four intermediate reaches (IJ 4, IJ 2, IJ 1 and IJ 5), and three heterogeneous reaches (IJ 7, IJ 3 and IJ 6). All stretches in the Witte Nete are homogeneous, and most stretches of the Kleine Gete are intermediate (except for the heterogeneous reach KG 6). The Desselse Nete is highly heterogeneous (Fig. 1; Table 1).

## Fish community

In total 23 fish species were captured in the four river zones. Between 11 and 15 species were caught in every river, with a total of 3–13 species per stretch (Fig. 2; Table 3). The species composition within every river (except for the Desselse Nete) was not equally distributed as shown in Fig. 3. Especially within the IJse and the Kleine Gete species composition between adjacent river stretches varied severely. For the Witte Nete, only the first and last stretch differed from the other stretches. Most stretches of the Desselse Nete were grouped, suggesting minor differences in species composition within the river zone.

In order to detect if homogeneous river stretches were supporting proportionally more locally 'rare' species than heterogeneous stretches do, a one-way

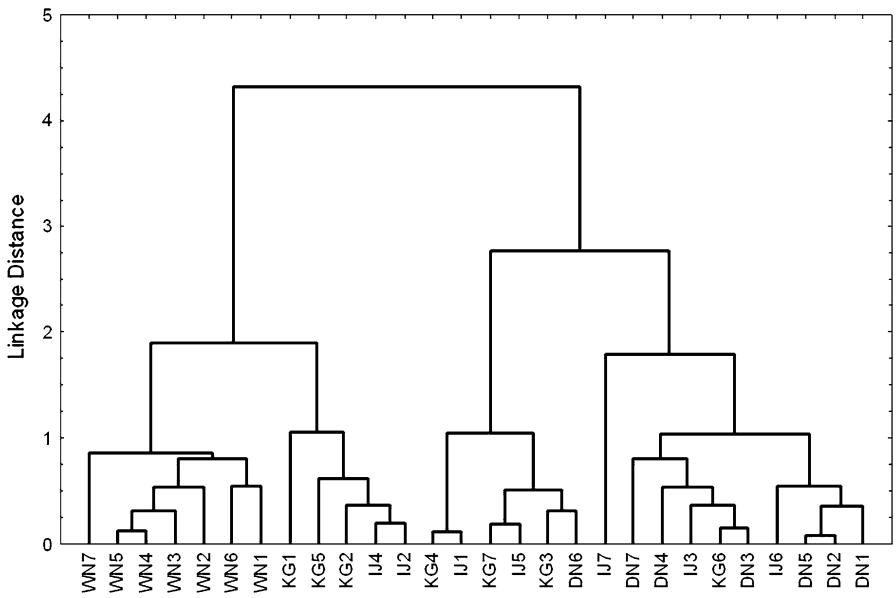


**Fig. 1** Cluster analysis (Tree diagram) for heterogeneity, using weighted pair-group average Euclidean distances for macro- and microhabitat characteristics (Habitat structure and depth diversity $CV_{DD\ cum}$)

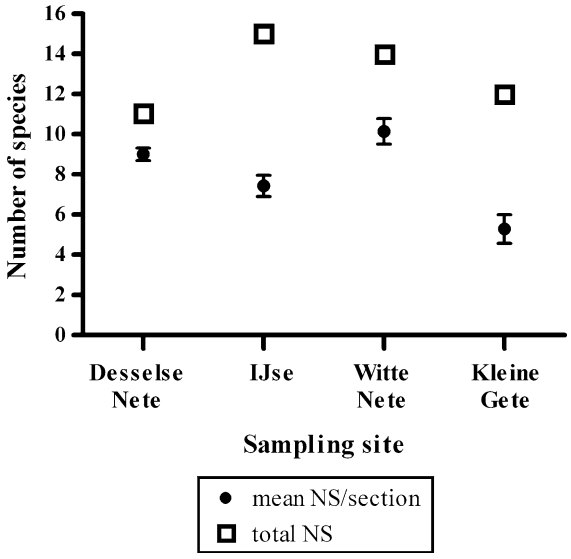


**Fig. 2** Total number of species of the four water courses, and mean number of species (with SD) caught within each single river stretch

ANOVA (W = 0.95) was conducted using the three habitat diversity groups described in "Habitat and depth diversity". In heterogeneous stretches, 24.5 ± 15.2% (SD) of the species present consisted of only 1 or 2 specimens. In intermediate and homogeneous stretches, these percentages are 31.5 ± 16.2 and 42.5 ± 20.3%, respectively. In homogeneous stretches, up to 60% of the species present consisted of locally rare species. The results show no real significant differences between homogeneous and heterogeneous stretches, but a trend could be noticed ($F_{2,25} = 2.613$; $P = 0.09$). However, the post-hoc comparison did not show significant differences between groups.

## Sampling distances

### *Fish species*

The results of the randomisation are summarised in Table 4. The regressions derived from the cumulative percentage of caught fish species related to the sampling distances are shown in Table 5. A logarithmic regression through all data points (Fig. 4) resulted in following model ($r^2 = 0.68$): % of caught species = 19.56 ln (sampling distance) − 28.02.

The overall regression was calculated to derive general conclusions on the minimal sampling distances required. This model revealed that in Flemish wadable rivers, a sampling distance of 250 m is necessary to collect 80% of the species present, 417 m to collect 90% of the species and even 539 m to collect 95% of the fish species. The regression was also calculated for homogeneous (Witte Nete and Kleine Gete) and heterogeneous rivers (IJse and Desselse Nete; Table 5).

### *Index of Biotic Integrity*

In Table 3, IBI classes (following Belpaire et al., 2000) of the adjacent stretches in the four rivers are shown: in all cases ecological integrity was allocated to scoring classes 3 (moderate) or 4 (good). Within river zones, variation in IBI-class occurred in the IJse and the Kleine Gete. In each case, a difference of only one IBI-class was observed between stretches. The IBI for the Desselse Nete and the Witte Nete is more stable, no changes in the IBI between adjacent reaches were observed. Since IBI-variation could possibly be induced by small differences in habitat diversity, as previously described, we tried to detect correlations between the number of species and habitat diversity ($r^2 = 0.02$, $P = 0.90$) and the number of species and IBI score ($r^2 = 0.26$, $P = 0.17$). Neither such correlations occurred, nor did any correlation occur between habitat diversity ($CV_{DD\ cum}$) and IBI score ($r^2 = 0.29$, $P = 0.13$). It is worth mentioning that the within group correlation test (river by river) showed that a positive correlation between IBI score and habitat diversity ($r^2 = 0.64$, only for the Kleine Gete). For the IJse, a significant correlation between habitat diversity ($CV_{DD\ cum}$) and the number of species ($r^2 = 0.85$, $P = 0.016$) was detected. Moreover, a positive significant correlation was found in the IJse between habitat diversity ($CV_{DD\ cum}$) and %NSP ($r^2 = 0.85$, $P = 0.016$). No further correlations were found.

# Discussion

## Fish community

Because habitat preferences often change during the course of development not only diadromic species show migratory behaviour, but also potamodromic species can undertake significant migrations to a

**Table 3** Species captured at each of the 28 sampling stretches on four Flemish brooks with their IBI evaluation (5 = no anthropogenic influence, 1 = bad status, Belpaire et al., 2000) with $NSP_{RS}$ = number of species in the river stretch and $NSP_{RZ}$ = species richness in the entire river zone

| Species | Desselse Nete | | | | | | | Witte Nete | | | | | | | IJse | | | | | | | Kleine Gete | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| *Abramis brama* | | | | | | | | | | | | | | | | | | | | X | | | | | | | | |
| *Anguilla anguilla* | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | | | | | | | | | | |
| *Barbatula barbatula* | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | | | X | | X | X | X | X | X | X | X | X |
| *Carassius auratus gibelio* | | | | | | | | | | | | | | | | | | | | X | X | | | X | | X | | X |
| *Cobitis taenia* | X | X | X | | X | X | X | X | X | X | X | X | X | X | | | | | | | | | | | | | | |
| *Cottus rhenanus* | | X | X | X | | X | | X | | | X | X | X | X | | | | | | | | | | | | | | |
| *Cyprinus carpio* | | | | | | | | | | | | | | | | | | X | | | X | | | X | | | | |
| *Esox lucius* | | | | | | | | | | | | | | | | | X | | | | | | | | | | | |
| *Gasterosteus aculeatus* | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | | | X | | | X | X | X | X | X | X | X |
| *Gobio gobio* | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | | X | X | X | X | X | X |
| *Gymnocephalus cernua* | | | | | | | | X | X | X | X | X | X | X | | | | | | | | | | | | | | |
| *Ictalurus nebulosus* | | | | | | | | X | X | X | | X | X | | | | | | | | | | | | | | | |
| *Lampetra planeri* | X | X | X | X | X | X | X | | | | | | | | | | | | | | | | | | | | | |
| *Lepomis gibbosus* | X | | | | X | | | | | | | | X | | | | X | | | | | | | | | | | |
| *Leuciscus idus* | | | | | | | | X | | | | | | | | | | | | | | | | X | X | | X | |
| *Oncorhynchus mykiss* | | | | | | | | | | | | | | | | | | | | | | | | | | | | X |
| *Perca fluviatilis* | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | | | | | | | X |
| *Pungitius laevis* | X | X | X | X | X | X | X | | X | X | X | X | X | | | | | | | | X | | | | | X | | |
| *Rutilus rutilus* | X | X | | | | | | X | | | X | | X | | | X | X | X | X | X | X | X | | X | X | | | |
| *Salmo trutta fario* | | | | | | | | | | | | | | | X | X | X | X | X | | X | | | | X | X | X | X |
| *Scardinius erythrophthalmus* | | | | | | | | | | | | | | | | | | | | X | X | | | | | | | |
| *Squalius cephalus* | | | | | | | | | | | | | | | | | | | | | | | | | X | | | |
| *Tinca tinca* | | | | | | | | | | | | X | X | | | X | X | X | | X | X | | | | | | | |
| $NSP_{RS}$ | 10 | 10 | 9 | 8 | 9 | 9 | 8 | 11 | 9 | 9 | 10 | 11 | 13 | 8 | 6 | 8 | 8 | 7 | 6 | 7 | 10 | 3 | 3 | 7 | 7 | 6 | 5 | 7 |
| $NSP_{RZ}$ | 11 | | | | | | | 14 | | | | | | | 15 | | | | | | | 12 | | | | | | |
| 100 * $NSP_{RS}/NSP_{RZ}$ | 91 | 91 | 82 | 73 | 82 | 82 | 73 | 79 | 64 | 64 | 71 | 79 | 93 | 57 | 40 | 53 | 53 | 47 | 40 | 47 | 67 | 25 | 25 | 58 | 58 | 50 | 33 | 58 |
| *IBI score* | 3.1 | 3.1 | 3.1 | 3.0 | 2.8 | 3.0 | 3.0 | 2.8 | 2.8 | 2.6 | 3.4 | 2.9 | 2.6 | 2.8 | 3.5 | 3.9 | 3.8 | 3.9 | 3.8 | 3.3 | 3.6 | 2.8 | 3.0 | 2.8 | 3.8 | 3.1 | 3.8 | 2.8 |
| *Ecological status (IBI -class)* | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 4 | 4 | 4 | 4 | 3 | 3 | 3 | 3 | 3 | 4 | 3 | 4 | 3 |

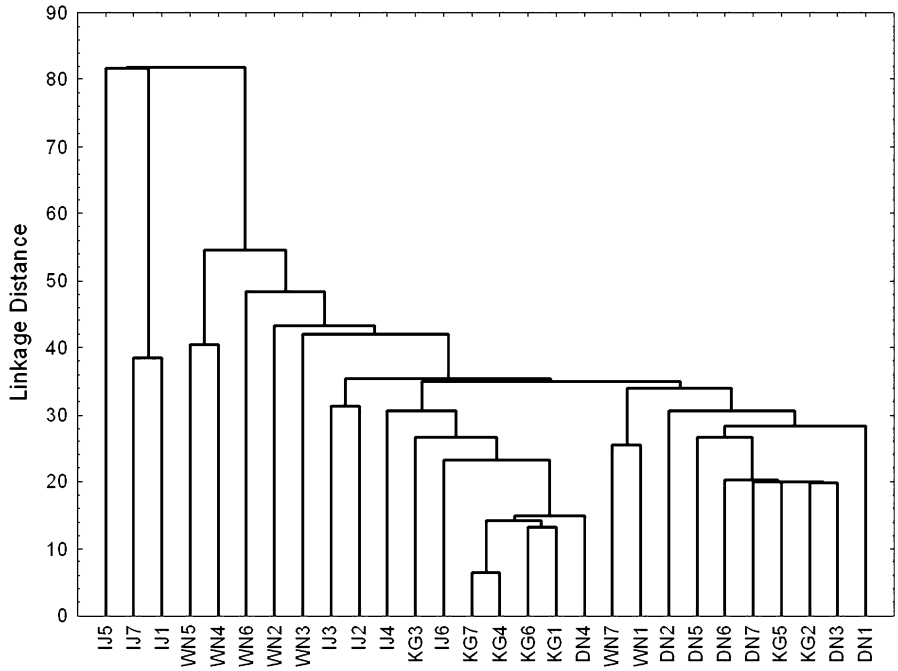


**Fig. 3** Cluster analysis (Tree diagram) for species composition (including specific proportions of species) using Single Linkage Euclidean distances

**Table 4** Cumulative % of caught species (% $NSP_{RZ}$ ± SD) in function of increasing sampling distance for the Desselse Nete, IJse, Kleine Gete and the Witte Nete in a 1,000 m river zone

| | 100 m | 200 m | 300 m | 400 m | 500 m | 600 m | 700 m |
|---|---|---|---|---|---|---|---|
| Desselse Nete | 81.8 (±6.9) | 90.0 (±9.7) | 94.6 (±11.2) | 97.4 (±12.2) | 99.1 (±12.7) | 100 (±13.0) | 100 (±13.0) |
| IJse | 49.5 (±8.6) | 67.3 (±12.8) | 77.9 (±15.2) | 85.5 (±17.0) | 91.4 (±18.4) | 96.2 (±19.4) | 100 (±20.0) |
| Kleine Gete | 44.1 (±14.6) | 61.9 (±18.9) | 73.1 (±21.0) | 81.4 (±22.3) | 88.1 (±23.2) | 94.1 (±23.9) | 100 (±24.6) |
| Witte Nete | 72.5 (±11.1) | 83.3 (±14.4) | 89.0 (±16.0) | 92.7 (±16.8) | 95.6 (±17.3) | 98.0 (±17.7) | 100 (±18.0) |

**Table 5** Regressions derived from the cumulative percentage of caught fish species related to the sampling distance, with the necessary sample length (m) to collect 80, 90 and 95% of the total number of species present

| River type | Regression | $r^2$ | 80% (m) | 90% (m) | 95% (m) |
|---|---|---|---|---|---|
| Overall | % of caught species = 19.56 ln (sampling distance) − 28.02 | 0.68 | 250 | 417 | 539 |
| Heterogeneous rivers | % of caught species = 17.86 ln (sampling distance) − 16.10 | 0.64 | 217 | 380 | 503 |
| Homogeneous rivers | % of caught species = 21.25 ln (sampling distance) − 39.94 | 0.75 | 282 | 452 | 572 |

larger or lesser extent (Grenouillet et al., 2000; Ovidio & Philippart, 2002; De Leeuw & Winter, 2008). These migrations are often more pronounced in homogeneous river stretches (De Leeuw & Winter, 2008). Seegert (2000) considered fish migration as a problem for fish stock evaluation and suggested to sample in periods when migration is low. However, several authors describe the phenomenon of fish movement (e.g. Hohausová et al., 2003; Heermann & Borcherding, 2006). Therefore, even besides spawning migration, fishes can be very mobile in search for food or shelter or migration could even be related to energy conservation of fishes (Heermann & Borcherding, 2006), meaning that the suggestion of Seegert (2000) is not sufficient to cope with natural fluctuations in fish communities. Bruylants et al. (1986) mentioned that fishes in homogeneous river stretches are more mobile than in heterogeneous reaches. Therefore, it is possible that certain species are not always sampled during consecutive fishing

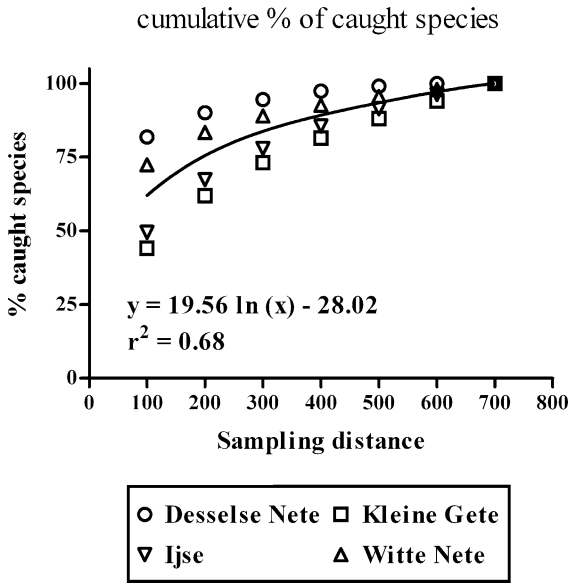


**Fig. 4** Cumulative percentage of caught fish species related to the sampling distance

periods, even when they are present in the water system or even within the watercourse itself. This could suggest that the standardised 100 m samples as presently used in several monitoring programs (including the Flemish one) do not always give a complete overview of the fish community present. Inadequate sampling will result in underestimation of species richness due to patchy distribution and spatial variation of stream fishes amongst habitats (Didier & Kestemont, 1996). Therefore, the range of sampled habitats plays a major role in data collection (Karr et al., 1986). The chance to capture locally rare species will increase when several scattered micro-habitats are included. The CEN standards suggest that the area of the river to be sampled is dependent upon width and habitat variation. Small streams (<5 m) should be sampled at least for 20 m; sample length of small rivers (5–15 m) should be at least 50 m. In order to ensure that conclusions on abundance and age structure are valid, a sufficient number of sites must be included. This number depends on the spatial variation amongst sites (CEN, 2003). Breine et al. (2005) stated that to ensure accurate characterisation of a fish community, electric fishing at a given site must be conducted over a river length of 10–20 times the river breadth, with a minimum of 100 m. In large and shallow rivers (width >15 m), several sampling areas covering in total at least 1,000 $m^2$ should be prospected. In a recent study, Hughes & Herlihy (2007) found that a sampling distance equal to 50 times that of the wetted channel width gave a stable index value.

In our study, we found that at least a distance of 250 m is necessary to collect 80% of the species present in a 1,000 m river zone. The sample distance increases to more than 500 m to collect 95% of the fish species. In contrast to the other rivers, in the Desselse Nete 83% of the species present was already caught after one sampling of 100 m. Moreover, there was even less variation between consecutive samples. These differences could possibly be explained by the high habitat diversity or heterogeneity of the Desselse Nete. It seems that all possible habitat structures are present in a 100 m river stretch, therefore every species could find several optimal habitats (feeding areas, hiding places, etc.) within a short distance. This theory is also supported by our findings that in homogeneous rivers more 'locally rare species' occur and that the proportion of total species caught in a river stretch seems to be positively correlated with habitat diversity.

It is possible that presumably non-migratory, sedentary fish species with different micro habitat characteristics for juveniles and adults (e.g. Van Liefferinge et al., 2005, 2006) are therefore able to complete their life cycle within a small reach of the river. In heterogeneous streams, intraspecific as well as interspecific competition can decrease because species can actively search for different microhabitats (e.g. Kadye & Magadza, 2008; Schwartz & Herricks, 2008). In very homogeneous reaches, fish are tend to be discontinuously distributed, tied to some sort of variation in the water course like an occasional deeper pool, overhanging riparian vegetation, bushes, branches, fallen trees,… where fish will congregate. For eurytopic and rheophilic species, the availability of sheltered diversified habitats with a diverse food supply is essential as nursery and feeding grounds (Karr & Dudley, 1981). A decrease of natural river systems or habitat diversity will therefore be reflected by an impoverished fish diversity (Brooks et al., 2004; Sindilariu et al., 2006; Lester & Boulton, 2008) or native species richness (Koel, 2004; Tales & Berrebi, 2007) often related to the decline of rheophilic species (Kruk, 2007; Maloney et al., 2008; Slawski et al., 2008). The presence of these structures is therefore crucial in homogenous reaches but structures are different and vary markedly

between stretches (Teels, 2003; Pusey & Arthington, 2003). This variability is influencing both the caught as well as the fish community present, suggesting that the sampling distance should at least partially be related to the present habitat structure. Moreover, different studies demonstrated that a 100 m section is not always sufficient to account for discontinuity in fish composition (Angermeier & Smogor, 1995; Paller, 1995; Patton et al., 2000, Teels, 2003). Accordingly, care should be taken not to misinterpret fish species composition or relative abundance when sampling effort has been too small.

It is not easy to provide definitive guidelines for what river length should be sampled to yield an accurate proportion of the local species present. In order to ensure the quality of data, collection methods must be standardised and a sample must accurately reflect the fish community present in a river stretch at a specific time. For one, sampling should include the minimum home-range sizes of the (dominant) species. This increases the probability that population variability is dominated by mortality and recruitment, rather than by movement in and out of the sample site (Grossman et al., 1990). The effects of disturbances may be masked by the natural variability of lotic fish assemblages (Grossman et al., 1990).

Moreover, the area required to collect all species must be sufficiently large to include both rare habitats and rarely occurring species (Paller, 1995). The necessary sample length depends on region and habitat diversity to capture the maximum number of species present in a certain river (Lyons, 1992; Angermeier & Smogor, 1995; Paller, 1995). This means that the sampling effort should depend on habitat diversity. However, in the case of long-term monitoring studies, a standardised protocol should be developed, so that accurate comparisons can be made across seasons or years.

In terms of time and effort, it is more efficient to sample a large area with a single-pass removal procedure than to sample a smaller area with many passes to collect the same number of species (Paller, 1995). Moreover, Reid et al. (2009) showed that single-pass surveys do provide a representative sample of species diversity of a certain river stretch. Bearing this in mind, we suggest a sampling reach of 300 m to sample most species present with a single-pass sampling method. If the aim of the study is to see which species are present in a particular brook or river or even species richness, a longer study reach (ca. 500 m) is necessary to collect all species present (or at least 95%).

Our results are slightly higher than the findings of other authors (Lyons, 1992; Paller, 1995; Patton et al., 2000). This could possibly be explained by the conducted sampling protocol using 100 m long sample sections within a river zone, which could lead to an overestimation of sampling needs. If sampling stretches had been shorter than 100 m, a shorter distance of stream may have been sampled to capture 90 or 100% of the species present. This means that estimates of sampling needs may be higher than the values that would have been obtained if shorter units had been used, but these sections were also used to evaluate their impact on the IBI. On the other hand, Paller (1995) stated that these long reaches are needed to represent all species including sporadically occurring species. In our study, relatively high proportions of locally rare species were found in homogeneous (42.5%) and even heterogeneous stretches (24.5%), which could at least partially justify the longer distances.

## Index of Biotic Integrity

In order to be capable to distinguish anthropogenic from natural disturbance, it is necessary to account for discontinuity in fish composition (Angermeier & Smogor, 1995; Paller, 1995; Patton et al., 2000; Teels, 2003). The size-of-area problem is often addressed by sampling mesohabitat and microhabitat types within the stream reach to catch all species present. However, caution is required to sample all habitats in proportion to their actual occurrence. The approach of sampling several run-riffle–pool habitat complexes is very impractical and very difficult when clearly recognisable habitat types are lacking, as it is often the case in low-gradient sand bottom streams in Flanders.

The structure of fish communities is typically characterised by species composition and proportional abundance. Accurate estimates of these parameters are essential because they are critical components of the assessment of ecological integrity (Karr et al., 1986). Since ecological health is based on biological diversity, the total number of species is a very crucial parameter for estimating biological integrity, especially because Osborne et al. (1992)

found significant correlations between IBI scores and the number of fish species.

Thus, for assessment of fish communities in function of monitoring programs, it is necessary to accurately estimate the ecological quality of the river stretch and to detect trends over time.

The value of a method quantifying ecological integrity depends on its usefulness in assessment. The IBI is expected to be capable to do so. Karr et al. (1987) concluded that the IBI consistently ranked sites according to assessments of site quality in streams where anthropogenic changes in water quality, and habitat were not evident through time. However, a useful index should be consistent over time at a site if no change in quality occurs. River stretches with a high degree of habitat diversity (higher quality sites) are less variable through time than lower quality sites due to the higher stability in fish community structure and the presence of habitat refuges (Karr et al., 1987). It seems that natural streams support fish communities of high species diversity, which are seasonally more stable than the lower diversity communities of modified streams. Seasonal changes in stream quality are high in modified streams whereas natural streams have more buffering capacity (Gorman & Karr, 1978).

The usefulness of an index is, therefore, a result of not only the sampling itself but also of the natural (seasonal) variation in fish composition. Increases in sampling effort yield diminishing returns in information on community attributes, such as number of species. Therefore, the most appropriate sampling effort should generally be the minimum effort that will provide the required information. The optimal effort depends on the study objective, but in the case of monitoring accuracy for instance, estimates of community attributes are crucial to allow meaningful comparisons. Comparing estimates based on (too) small sampling effort should be avoided because real differences in community structure may not be distinguishable from biases due to inaccurate sampling. An ecological change over time that exceeds the range of normal variability together with the direction of change, can reinforce the conclusion that disturbance is present, and indicate whether a stream is recovering or deteriorating.

The IBI which is used for monitoring studies is strongly affected by rare species, which tend to be discontinuously distributed. Sampling effort should be high enough to catch these rare species, which could be present in the study reach. This is especially true for homogeneous streams, considering that in our study on average 42.5% (sometimes even up to 60%) of the species diversity in a river stretch consisted of locally rare species.

The sampling effort necessary to accurately characterise fish community structure is inversely related to population density, which may be associated with habitat unit homogeneity for most species (Angermeier & Smogor, 1995). This pattern suggests that homogeneous river stretches are supporting proportionally more locally 'rare' species than do heterogeneous stretches. Our results seem to support this theory since they show this trend even with relatively low numbers of real homogeneous and heterogeneous river stretches. Furthermore, our data show a positive correlation between habitat diversity and number of species. This suggests that the number of fish species caught with the same sampling effort is likely to be the lowest in homogeneous stretches compared to heterogeneous stretches. In order to accurately assess the fish population and species richness, the ecological evaluation of the river ecosystem should be reflected in the assessment. Hence, it is important not to oversize the sampling effort only to catch more species.

Information on spatial distribution patterns of fishes and species richness is important for the conservation and management of fishes. In bio-monitoring programs (at population level), it is very important to distinguish substantial ecological changes from normal background fluctuations in fish assemblage structures. Normally, the IBI is quite stable when water quality remains approximately the same (Karr et al., 1987). IBI scores are, therefore, reproducible when no changes in habitat or water quality were evident. However, attention should be drawn to the fact that there is greater fish assemblage persistence and stability at undisturbed, pristine sites than at disturbed sites. Changes of physical habitat structure are likely to occur more frequently at undisturbed sites, which can result in temporal variability in fish assemblage structure (Paller, 2002). Grossman et al. (1990) pointed out that stream fish communities do exhibit a high variability, suggesting that it may be difficult to detect the effects of anthropogenic disturbances using population data alone. Consequently, monitoring fish assemblages as indicator of aquatic health requires caution. Collection methods must be

standardised to ensure the quality of the data, and a sample must accurately reflect the fish community present in a stream reach at a specific time (Karr et al., 1986). An important criterion for such bio-monitoring programs is to achieve low variability within the measures, therefore sampling has to be precise and adequate. Accurate sampling requires the prospection of a variety of meso-habitats for the evaluation of river quality (Didier & Kestemont, 1996). Therefore, one can presume that sampling a reach length of more than 100 m will allow monitoring to distinguish between anthropogenic disturbance and natural variation with greater reliability. However, we found a correlation between habitat diversity and IBI score, suggesting that habitat diversity could be responsible for changes in IBI scores. Moreover, no changes in IBI were observed between individual stretches in the Witte Nete (homogeneous river zone) and the Desselse Nete (heterogeneous river zone) suggesting that especially in river zones classified as intermediate (Kleine Gete, IJse) variability in assessments of ecological integrity occurs. During our sampling sessions, no visible changes in physicochemical water quality occurred in the different river stretches. Therefore, the IBI could effectively detect differences in between consecutive stretches, which were induced by small differences in habitat diversity (at least for the Kleine Gete). Therefore, if the original aim of the monitoring program is to determine the biotic integrity of a given river stretch, a 100 m sample section could be enough to determine the ecological integrity accurately, in sharp contrast to the sample distance necessary to capture all species (including locally rare species) present.

## Conclusion

Fish stock assessments need to be standardised to ensure the quality of the data, accurately reflecting the fish community present in a river zone at a specific time. However, depending on the aim of the study different protocols can be used. In the case of determining species richness of a given river zone, our results show that a mean sample distance of 250 m is necessary to collect 80% of the species present, 417 m to collect 90% of the species and even 539 m to collect 95% of the fish species. However, when the major goal of the fish stock assessment is to monitor the ecological status as prescribed in the Water Framework Directive, one needs a reliable estimation of the ecological quality of a given river zone. Apparently, sampling a 100 m river stretch as presently applied in the Flemish monitoring programs can accurately describe the ecological quality. Differences in IBI score between adjacent river stretches in a river zone could be explained by small differences in habitat heterogeneity. Therefore, we can conclude that electric fishing over a 100 m river stretch accurately reflects the ecological integrity based on fish communities for all wadable streams and rivers. However, caution is required to use the findings of this study for larger river systems. More appropriate fishing techniques (cfr. CEN, 2005) should be used to accurately describe their species richness and hence their ecological quality.

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